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Supplemental information

Mining HIV controllers for broad and functional antibodies to recognize and eliminate HIV-infected cells

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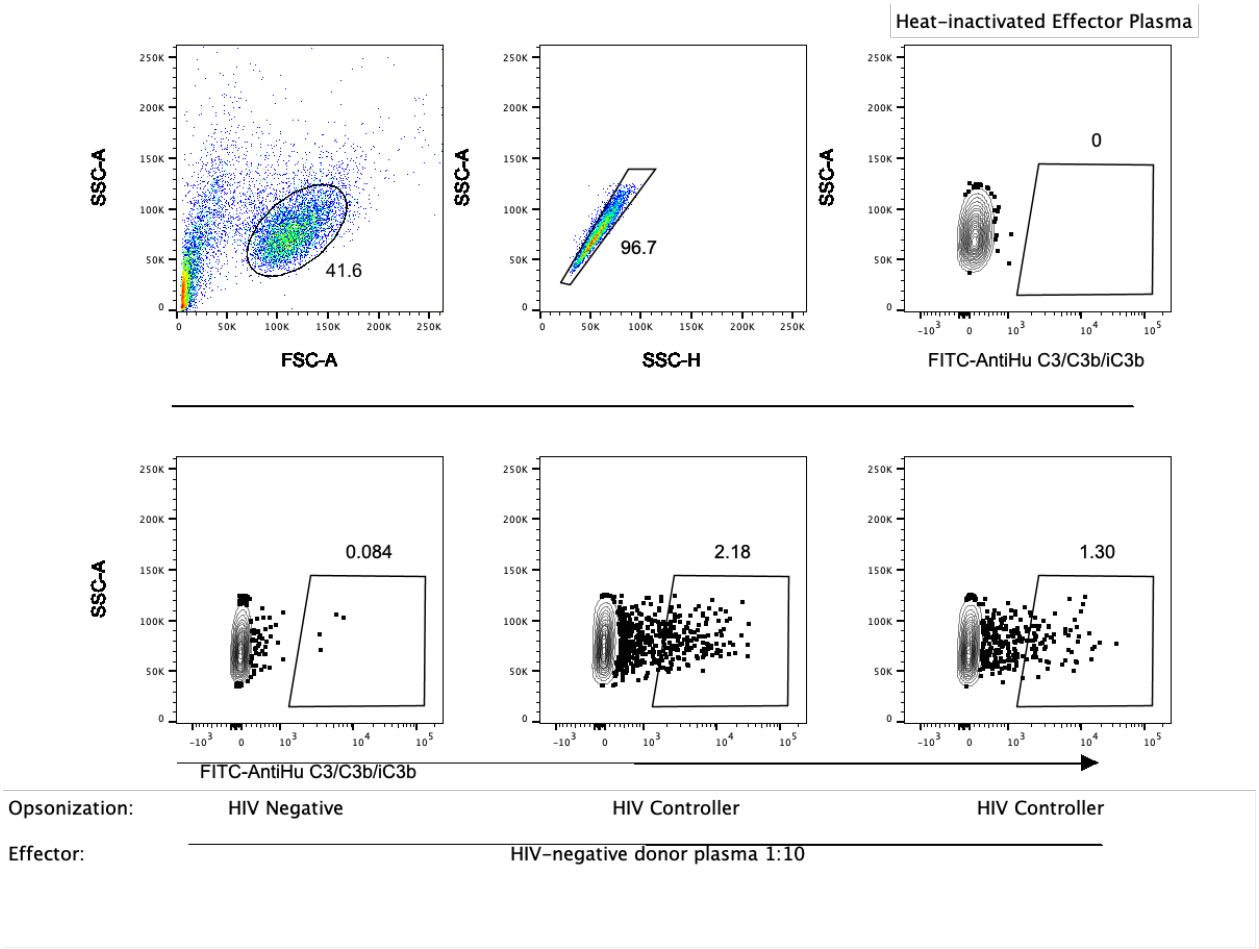

Figure S1: Antibody-dependent complement deposition on the surface of antigen-pulsed CEM-NKr-CCR5 lymphocytes. Figure S1 is related to Figure 1. CEM-NKr-CCR5 lymphocytes were pulsed with gp120 YU-2, washed, and opsonized with heat-inactivated human plasma, before the addition of complement source. **A)** Gating scheme for complement deposition in the absence of active complement (heat-inactivated human serum). **B)** Comparison of complement deposition levels (gated on single cells) by HIV- plasma (negative control, left) and HIV controller plasma (right) in the presence of human complement. Complement+ gate was set by heat-inactivated plasma levels (A, right). Numbers show the per cent frequency of events within the gate among the parent gate.

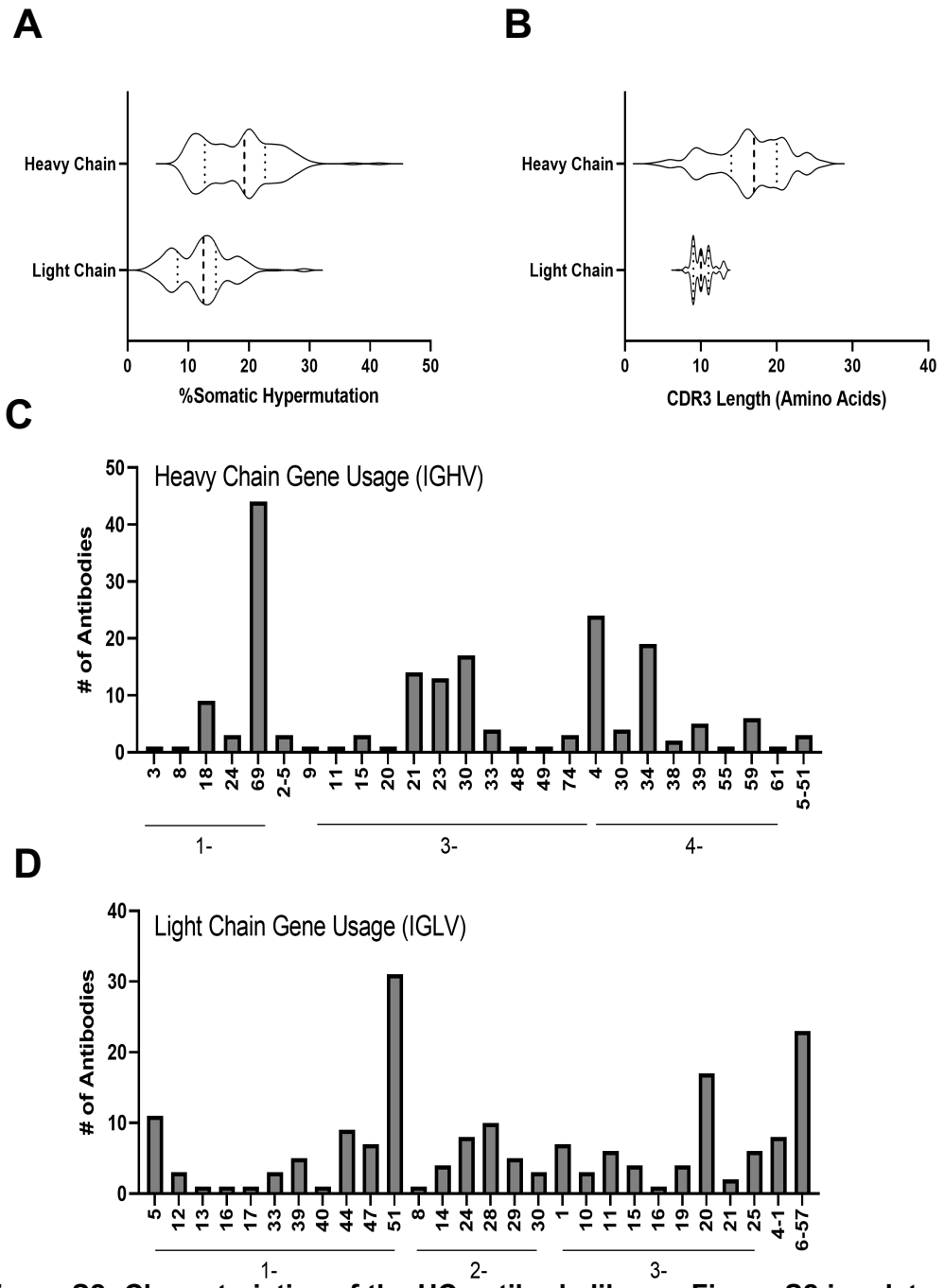

Figure S2: Characteristics of the HC antibody library. Figure S2 is related to Figure 1. (A) Somatic hypermutation rates are shown here as frequency of divergence of the antibody heavy chain compared to the inferred germline sequences. (B) The length of the CD3 variable region in the heavy and light chains in amino acids. The frequency of inferred germline usage among the heavy (C) and light chains (D). In the violin plots (A&B), the median value is depicted as a dashed line, with the 25th and 75th quartiles as dotted lines.

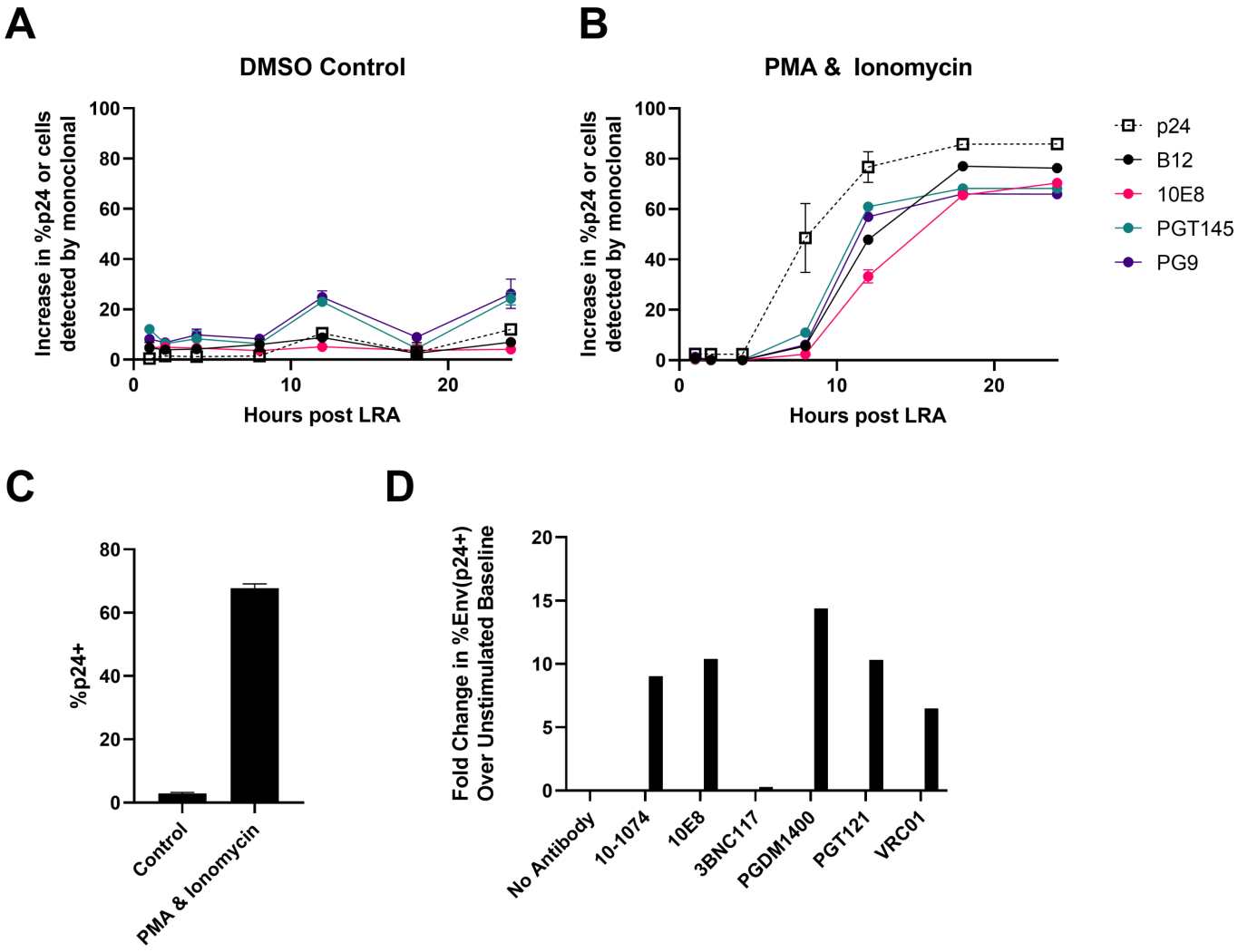

Figure S3: Ability of bNAbs to recognize reactivated latent cells. Figure S3 is related to Figure 2. Latency model ACH-2 cells were incubated in the presence of (A) DMSO (Control) or (B) PMA/Ionomycin (P/I) and the ability of bNAbs to detect extracellular epitopes on reactivated (p24+) cells, as quantified by flow cytometry, is displayed over a time course of 24 hours. p24 was measured by intracellular staining. Latency model J89-GFP were treated with DMSO or P/I for 18 hours and tested for reactivation (p24+) (C) and the baseline-subtracted fold-change in recognition by bNAbs after stimulation (Env+p24+). Values are the $(\%Env+(p24+)_{PMA} - \%Env+(p24+)_{No\ Stim})/ \%Env+(p24+)_{No\ Stim}$ (D). Data in A-D are the averages of triplicate values, error bars represent the standard deviation. PMA=phorbol myristate acetate, DMSO=dimethyl sulfoxide.

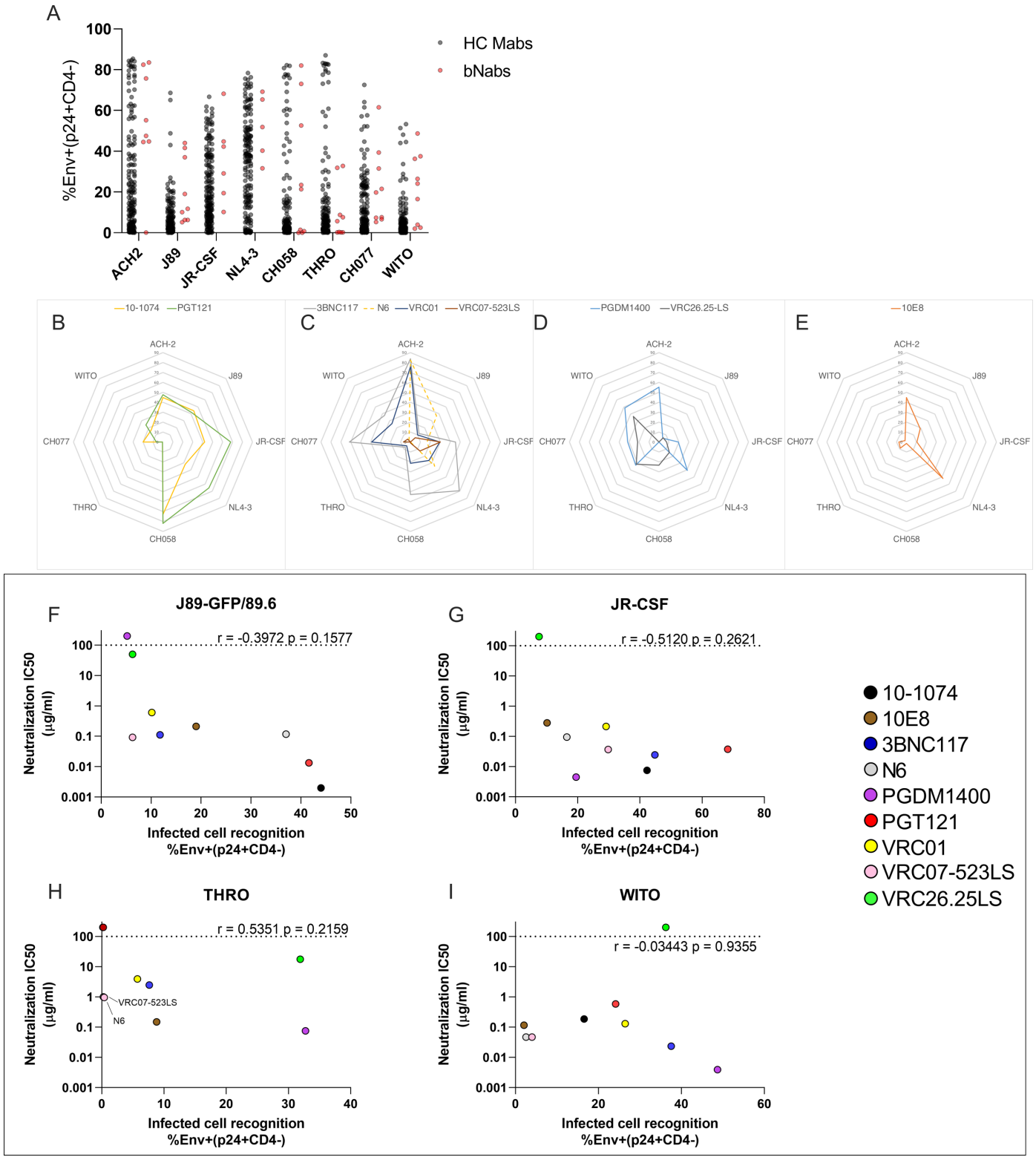

Figure S4: Recognition rates of infected lymphocytes by HC mAbs and bNAbs. Figure S4 is related to Figure 2. A) Group comparison of the recognition rate of 185 HC mAbs and the comparison of nine

bNAbs across different virus-infected cells. **(B-E)** The ability of individual bNAbs to bind cells infected with indicated viruses is shown in radar plot (summarized as a group in **A**). Antibodies are grouped by epitope, (B) V3-glycan, (C) CD4bs, (D) V1-V2, and (E) MPER. Antibodies were used at a concentration 25 μg/ml. Values indicate the percentage of infected (p24+) cells recognized by each antibody. (F-I) Correlations of infected cell binding with reported neutralization potency across different tested viruses. The geometric mean IC50 from reported studies (3,10,11,17,94–97) was obtained from CATNAP (98), and is displayed relative to our infected cell recognition data. Undetectable neutralization IC50 is displayed as >100 μg/ml. Viruses for which over half of the neutralization values were unavailable were omitted. Pearson correlations and significance is displayed.

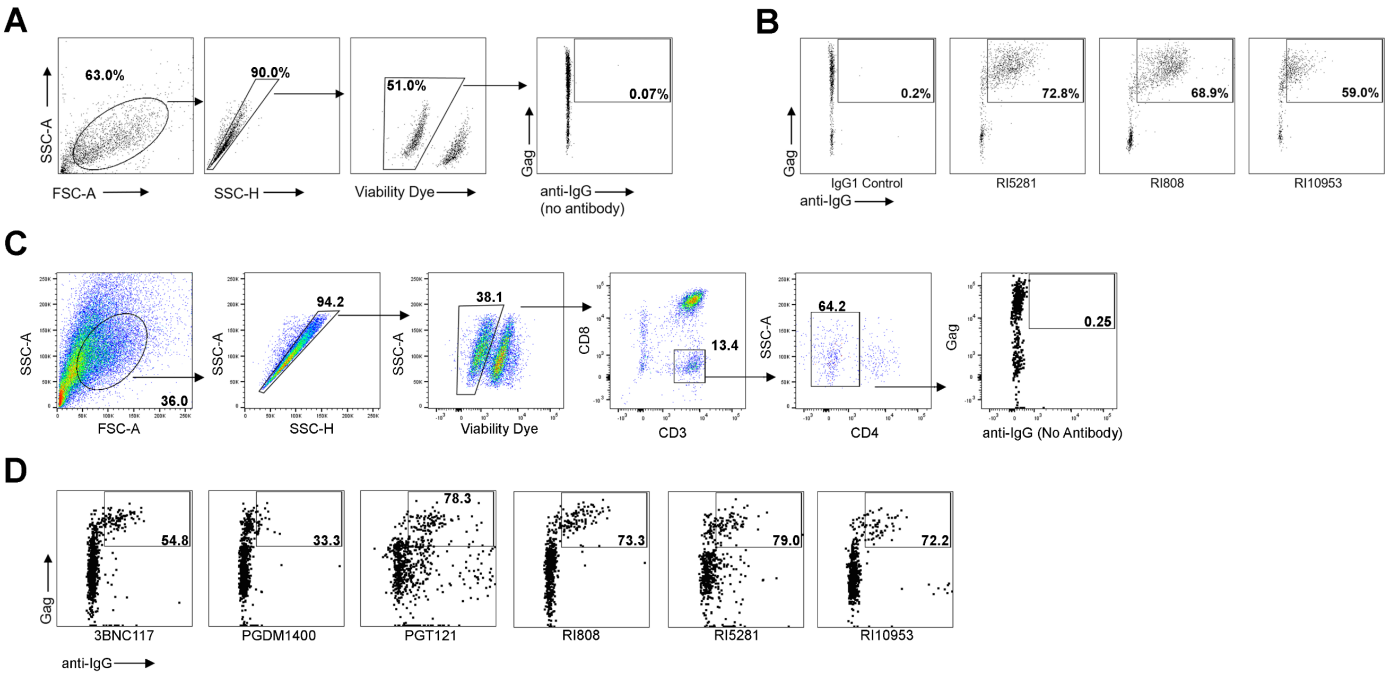

Figure S5: Recognition of infected lymphocytes by anti-Env antibodies. Figure S5 is related to Figure 2. (A) Gating scheme for Env recognition in JR-CSF-infected CEM-NKR-CCR5 cells. Cells are first gated based on size using on FSC/SSC, followed by doublet discrimination by SSC, and exclusion of dead cells using Blue Viability Dye. B) Characterization of monoclonal antibody binding to JR-CSF-infected CEM-NKR-CCR5 cells. Cells were opsonized with the indicated antibody, washed, and IgG1 binding was detected by a secondary antibody against human Fc. C) Gating scheme for Env recognition in PBMCS. Cells are first gated on FSC/SSC based on size, followed by exclusion of Blue Viability Dye, CD3+CD8-. Our analysis focuses on cells that have downregulated CD4. D) Characterization of monoclonal binding to HIV-infected primary cells. Numbers display the frequency of Env positive cells among infected (gag+) cells.

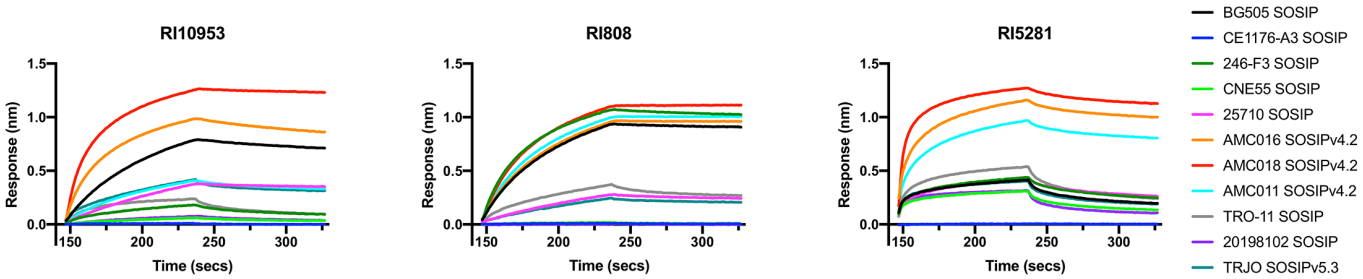

Figure S6: BLI sensograms showing association of top HC antibodies with SOSIP-trimers. Figure S6 is related to Figure 5.

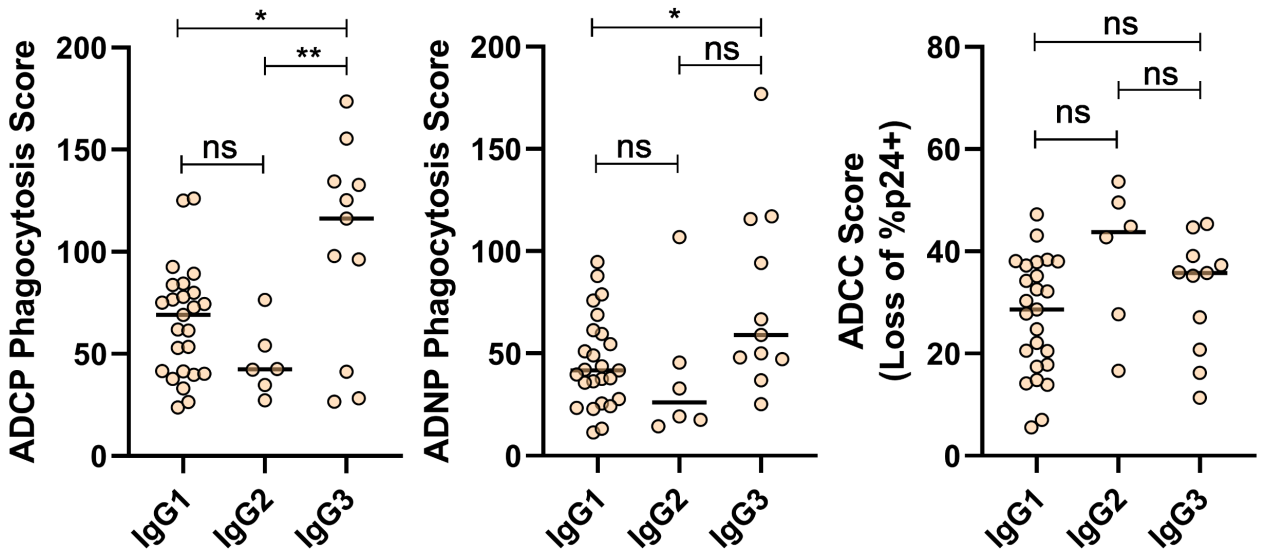

Figure S7: Association between the original isotype of sorted B cells and the Fc function of the recombinant IgG1 antibody among the downselected HC antibody library. Figure S7 is related to Figure 6. Fc-function data from Figure 6 are displayed, categorized by the isotype of the original B cell sort. Significance is calculated by one-way ANOVA corrected for multiple comparisons with Tukey's multiple comparison test. * $p<0.5$, ** $p = 0.0062$.

ID	Gender	Age at time of sampling	At time of sampling		
			Yrs since HIV diagnosis	CD4 T-cell count (cells/μL)	HIV RNA (copies/ml)
939369	Female	26	9	681	90
847041	Female	51	23	1769	ND
622800	Male	63	24	1096	65
534694	Male	59	6	661	184
504350	Male	48	18	925	466
447160	Male	61	22	639	ND
444154	Male	62	20	667	4540
437105	Male	64	24	749	1190
386576	Female	46	15	404	ND
330183	Male	44	4	1038	3180
315504	Male	57	22	427	ND
280008	Male	61	27	823	31
211774	Male	45	12	730	227
196203	Male	39	4	761	1510
191551	Male	47	18	421	1095

ND = Not detectable

Table S1: HIV Controller Characteristics. Table S1 is related to Figure 1.

Antibody	Functional Score	Glycan Species (%)														Grouped Total (%)		
		G0F	G1F	G2S1F	G1.F-G1FB	G2F	G2S2FB	G2S1	G1.FB-G2	G1S1F	G2S1B	G1B	G2FB	G1.-G0FB	G0	Afucose	Bisected	Sialylated
c2496	324.00	56.87	11.45	11.35	8.58	7.40	0.00	0.00	0.00	0.00	0.00	4.35	0.00	0.00	0.00	4.35	12.93	11.35
RI5281	255.70	53.92	19.71	6.27	7.48	4.50	1.04	0.00	0.00	0.69	0.00	0.44	0.57	1.71	0.84	1.28	11.24	8.00
VRC07-523 LS	245.76	55.51	29.29	0.00	9.28	5.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.28	0.00
c1144	235.78	50.50	14.05	9.84	6.13	3.82	0.00	7.36	4.24	0.00	4.06	0.00	0.00	0.00	0.00	7.36	14.43	21.26
c1369	206.99	82.51	11.03	6.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.46
10-1074	199.08	86.19	13.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RI808	177.17	81.57	18.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3BNC117	162.94	62.93	17.26	12.21	7.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.59	12.21
RI10953	142.42	87.62	12.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
c5260	135.81	61.17	22.47	0.00	7.04	4.45	0.00	0.98	1.19	0.00	2.03	0.00	0.00	0.00	0.66	1.64	10.26	3.01
c5272	130.99	45.95	13.29	27.20	4.70	0.00	5.81	0.00	0.00	3.04	0.00	0.00	0.00	0.00	0.00	0.00	10.51	36.05
c5266	115.81	84.98	15.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PGT121	108.58	76.46	13.88	9.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.66
c5232	87.11	68.71	19.05	2.08	7.13	3.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.13	2.08
PGDM1400	83.92	80.93	19.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
c5237	81.51	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
c5229	79.38	38.05	28.80	3.60	8.52	16.47	0.00	0.00	2.33	0.00	0.00	0.00	2.23	0.00	0.00	0.00	13.08	3.60
c5299	60.95	83.95	16.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Mean	157.44	69.88	16.39	4.93	3.69	2.53	0.38	0.46	0.43	0.21	0.34	0.27	0.16	0.10	0.08	0.81	5.36	6.32
bNAb Mean	160.06	72.40	18.66	4.37	3.37	1.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.37	4.37
HC Mean	156.43	68.91	15.52	5.14	3.81	3.05	0.53	0.64	0.60	0.29	0.47	0.37	0.22	0.13	0.12	1.13	6.12	7.06

Table S3: Fc glycosylation of monoclonal antibodies used in functional studies. Table S3 is related to Figure 6. Glycan analysis by capillary electrophoresis was performed on a selection of antibodies used in functional studies. Glycan species are displayed in percentage and organized from left to right in overall abundance. Antibodies are organized by aggregate functional score (sum of ADCP, ADNP, and ADCC, shown in Figure 6). The total percentage of afucosylated, bisected, and sialylated glycans are shown on the right. Afucosylated grouped total is the sum of G2S1, G1B and G0 peaks, bisected is the sum of G1.F-G1FB, G2SF2B, G1.FB-G2, G2S1B, G1B, G2FB, and G1.-G0FB, sialyated glycans are the sum of G2S1F, G2S2FB, G2S1, G1S1F, and G2S1B. For comparison the mean abundance of each glycan overall (Total) or within each group (HC or bNAb) is shown on the bottom.